1992

Leakage of Solutes from Imbibing Seeds of Supersweet Corn Cultivars That Differ in Tolerance to Low Germination Temperatures

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LEAKAGE OF SOLUTES FROM IMBIBING SEEDS OF SUPERSWEET CORN CULTIVARS THAT DIFFER IN TOLERANCE TO LOW GERMINATION TEMPERATURES

(TITLE)

BY

LINDA KULL

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
Masters of Science
Major in Botany

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1992
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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ABSTRACT

Supersweet types of sweet corn (Zea mays L. var rugosa) with shrunken-2 endosperm are preferred by consumers because of their extra sweetness and postharvest retention of good flavor. However, their acceptance by growers is hindered due to reduced field emergence, especially in cold soils. This reduced emergence is related to seed quality. To examine differences between supersweet corn cultivars which are tolerant and sensitive to low temperatures, two cultivars were chosen based on their emergence in the field at low temperatures (10-15 C). 'Illini Gold' was chosen as the tolerant cultivar and 'Honey'n'Pearl' as the sensitive cultivar. Seeds of both cultivars were germinated at 10, 15, and 20 C. Germination percentages and days to 25% germination were calculated. Seed leachate was analyzed for electrical conductivity, potassium, calcium, sugars, and amino acids.

At all temperatures germination percentages were higher for 'Illini Gold' than for 'Honey'n'Pearl. Germination percentages were higher at 15 and 20 C than at 10 C for both cultivars. Days to 25% germination was lower for 'Illini Gold' than for 'Honey'n'Pearl'. Percent ion leakage was 2.2 times higher for 'Honey'n'Pearl' than for 'Illini Gold' and higher at 20 C than at 10 C. At all temperatures, potassium, sugars, and amino acids in seed leachate were higher for 'Honey'n'Pearl' than for

'Illini Gold' and paralleled electrical conductivity. Imbibitional temperature effects were not significant for leakage of sugars and amino acids, and significant for potassium leakage. Calcium in seed leachate was less than 0.001 mg/seed/ml.

Imbibitional leakage of ions, potassium, sugars, and amino acids were more dependent upon cultivar differences than germination temperatures.

ACKNOWLEDGEMENTS

To my major professor Dr. Janice Coons, I wish to convey my heartfelt appreciation for the quality of guidance given to me during the past two years. Your integration of friendship and professionalism is meritorious. Thank you.

A warm thanks goes to my mentor, Dr. John Speer. You have a special way of sharing the diversity of knowledge and wisdom that you hold.

To my committee members, Dr. Janice Coons, Dr. John Speer, and Dr. Andrew Methven, a special thanks is extended for your time and suggestions toward the completion of this thesis. You are appreciated.

For taking time from a busy lab schedule, a cordial thank you is given to Dr. Jonathan Filley of the chemistry department. Your understanding of flame atomic spectroscopy aided in the completion of this project.

Bill, my husband and best friend, thank you most dearly for unwavering encouragement and support. Your enthusiasm for my interests deeply touches me.

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CHAPTER 1

INTRODUCTION

Fresh market supersweet corn cultivars have been developed which contain the shrunken-2 (sh-2) gene or the sugary enhancer (se) recessive gene modifier. These genes result in high sugar mutant endosperms. The sh-2 gene causes sweet corn kernels to contain 2 to 3 times more sugar at maturity than the traditional sugary (su) gene (Styer and Cantliffe, 1983A), but also results in reduced levels of phytoglycogen which impart a desirable creamy texture. The se gene modifier doubles endosperm sucrose content while maintaining high levels of phytoglycogen (Juvik and LaBonte, 1988). Sweet corn containing sh-2 and se genes are preferred by consumers because of their sweet taste and their postharvest retention of good flavor, but are not as widely accepted by growers as traditional sweet corn because of inferior seed quality, cold temperature susceptibility, and reduced germination (Tracy and Juvik, 1988).

Seeds that contain sh-2 and se genes for high sugar mutant endosperm exhibit a higher risk of pre-emergence failure than do seeds with traditional su genes. Specific causes of this increased failure rate are unclear. Close examination of the pericarp, which should restrict or regulate water uptake and metabolite leakage during imbibition, reveals that it is extremely shrunken, wrinkled, and has visible holes. The higher sugar endosperm content results in a higher osmotic potential

in supersweet seed relative to traditional seed (Styer and Cantliffe, 1983A). High osmotic potential and reduced pericarp integrity allows more rapid water uptake during initial phases of imbibition and increases the possibility for embryo damage or metabolite leakage (Styer and Cantliffe, 1983A). Rapid water uptake also interferes with cell membrane reorganization of dried seed and allows increased metabolite leakage from seed (Styer and Cantliffe, 1983A). Also, the leakage of sugars during imbibition encourages pre-emergence mortality due to rot by soil pathogens.

Germination of sweet corn seeds is influenced by both chemical and physical factors that are present during seed formation and seed germination. One of these factors is temperature, which affects germination, emergence, and vigor of sweet corn seed (Styer and Cantliffe, 1983B). In greenhouse and field cold soil tests using sh-2 seeds, only the most vigorous seeds emerged (Styer and Cantliffe, 1983B). Viability of sh-2 seeds in cold soil tests was much lower than that of su seeds (Styer and Cantliffe, 1983B). Styer and Cantliffe (1983B) deduced that the vigor of sh-2 seeds can be increased by production in a favorable environment, whereas the su seeds still produced high quality seeds regardless of the production environment.

The combination of genetic characteristics discussed for supersweet seeds and low temperature during imbibition causes metabolites to leak from seeds. In order to improve

quality of seeds with high sugar mutant endosperm, the composition of this metabolite leachate must be examined. This study focused on two supersweet corn cultivars, 'Illini Gold' and 'Honey'n'Pearl', which differ in their tolerance to low temperature. The following objectives were studied:

1) Determine percent germination and percent ion leakage at 10, 15, and 20 C of the two supersweet corn cultivars.
2) Analyze the seed leachate for soluble sugars, amino acids, potassium, and calcium after imbibition for 3 hours at 10, 15, and 20 C for the two supersweet corn cultivars.

CHAPTER 2

LITERATURE REVIEW

Corn (*Zea mays*, L.) probably originated in Central and South America where it developed as a warm season crop. Corn cobs were found in caves in Mexico dating as early as 5000 BC. The United States colonists began to grow corn for livestock in the mid-1700's, but it was not until the mid-1800's that sweet corn became a popular vegetable crop in the United States. In 1918, D.F. Jones produced corn hybrids which were first sold commercially in the 1930's. In 1951, Emil Wolf began work with the supersweet types of corn, and in 1974, supersweet corn seed was marketed. Today, corn is a major economic food crop, ranking as the fourth most important fresh vegetable crop in the United States. In Illinois the sweet corn industry harvests approximately 50,000 acres per year with fresh market production accounting for 5000 acres annually (Coons, 1986).

The appealing traits of sweet corn are controlled by mutant genes, located on chromosome 4 at the *su* locus. This mutation affects sweetness of flavor, texture, postharvest quality retention, and pericarp tenderness. Sweetness is controlled by 3 main genes: *sugary* (su), *shrunken-2* (sh-2), and *sugar enhancer* (se). The traditional *su* gene causes the sweet corn to be high in water soluble polysaccharides which produce a creamy texture, to be low in sugar (only 2 times that of field

corn), and to have a rapid postharvest conversion of sugar to starch (Coons, 1986). The sh-2 and se genes designate the supersweet corn description and cause the kernel to be low in water soluble polysaccharides, to have a high sugar endosperm content (3 times the amount of sucrose found in the traditional su corn types), and to retain its postharvest sweetness longer than that of the traditional sweet corn kernel (Coons, 1986). The postharvest retention of the relatively higher sugar endosperm content is a very desirable trait for long distance fresh market transport. Within 24 hours at 27 C postharvest sucrose content (% dry wt) for su kernels decreased by 60% and for sh-2 kernels decreased by 20 % (Garwood, et al, 1976).

Achieving successful germination and emergence from supersweet corn seeds planted in cold soils (i.e. 10 - 15 C) remains a challenge to growers and researchers. To ensure a top fresh market price for sweet corn, a warm season crop, it must be planted during the spring season when the potential for chilling injury is present. If planted later, the possibility of high summer temperatures interfering with pollination would result in lower yields and inferior kernel quality. To further understand the inherent and environmental factors affecting the acceptance and success of supersweet corn cultivars, additional studies involving cold temperature germination are required.

Low Temperature Germination and Chilling Injury

The success or failure at low temperatures of seed germination is influenced by conditions present during seed development, seed drying, seed harvesting and packaging, seed storage, imbibition, and radicle emergence (Bennett, Waters, and Curme, 1988). When all environmental factors influencing the success of germination are optimum, differences are still observed between cultivars due to genetic variability and crop origin. Based on low temperature sensitivity, species can be divided into tolerant or sensitive cultivars. Cultivars which are susceptible to chilling injury, a physiological disorder that occurs at temperatures between 0 and 10 to 15 C (Herner, 1986), originated in tropical or subtropical regions while cultivars resistant to chilling injury originated in temperate regions (Lyons, 1973).

Chilling-sensitive seed producing plants can be further divided into two groups based on the time at which chilling injury occurs at low temperatures (Herner, 1986). One group shows injury occurring if low temperatures are present after radicle growth has been initiated. These plants are not sensitive to chilling injury during imbibition which is prior to radicle growth (Herner, 1986). The second group of seed producing plants are injured if imbibition begins at low temperatures. Sweet corn is susceptible to chilling injury when

initially imbibed at low temperatures (Walters and Blanchette, 1983).

The factors that affect imbibitional chilling injury are: 1) temperature; 2) relation between timing of low temperature and stage of germination; 3) moisture content of seed before imbibition; 4) rate of imbibition of water; 5) seed coat integrity; 6) seed vigor; and 7) species or cultivar (Herner, 1986). Imbibitional chilling injury is greater if imbibition is initiated at low temperatures than if initiated at moderate temperatures (Pollack and Toole, 1966). If plants are in the group that are injured if imbibition begins at low temperatures, then little or no injury results if initially imbibed in warm conditions followed by low temperatures (Pollack and Toole, 1966). Surrounding seeds by high relative humidity to allow the seed moisture content to increase slowly protects seeds from chilling injury when initially imbibed at low temperatures (Cal and Obendorf, 1972; Obendorf and Hobbs, 1970).

Rapid rate of imbibition due to loss of seed coat integrity is related to poor germination at low temperatures. Seed coat cracking or removal causes increased imbibition rates and increased solute leakage as measured by electrical conductivity (Styer and Cantliffe, 1983A). The reduced amount of endosperm, as determined by species or cultivar genotypes, may account for rapid imbibition in seeds. This reduction results in accelerated hydration of the embryo and, therefore increases the potential for tissue damage (Styer and Cantliffe, 1983A).

Sweet corn kernel quality is influenced by conditions present during preharvest and postharvest kernel maturation. Cellular respiration utilizing corn kernel sugars continues with gradual decline throughout preharvest maturation of the kernel on the mother plant. This reduction coincides with the loss of seed moisture content, reduction in endosperm sugar/starch ratios, and increase in kernel dry weight (Styer and Cantliffe, 1983A). During postharvest maturation cellular respiration increases as temperature increases and rapidly reduces seed vigor. Studies show that 50% of the su kernel sugar content is lost 24 hours after harvest (Coons, 1986). From a consumer's perspective, increased cellular respiration in the kernel causes a decrease in kernel sweetness resulting in a less desirable product. The supersweet kernel contains 2 to 3 times more endosperm sugars than traditional types (Wann, 1986). Therefore, if 50% is lost, the supersweet kernel still retains a higher postharvest sugar content than does the traditional kernel.

When the factors that affect imbibitional chilling injury are investigated independently, each negatively influences seed vigor, seed quality, and germination success. Combinations of these factors, for example, low seed moisture, high sugar/starch ratios, and imbibition at low temperatures, are very damaging (Obendorf and Hobbs, 1970). Depending on cultivar type, supersweet corn seeds have many of the previously mentioned inherent factors and exhibit the consequence of poor

emergence, especially at low temperatures.

A number of hypotheses have been proposed to explain the causes of chilling injury during imbibition of seeds with warm climate origins. Based on investigations of seed anatomy and physiology during imbibition, suspected causes of chilling injury are: 1) leakage of ions or compounds from the imbibing seed; 2) high sugar/starch endosperm ratios; 3) pericarp cracking during the dehydration stage of seed maturation; 4) loss of membrane integrity; and 5) pathogen attack. Many causes of chilling injury during low temperature imbibition of warm season crops are not yet known. Low temperatures during imbibition of seeds with high sugar/starch endosperm ratios result in leakage of more electrolytes and other solutes than seeds with low sugar/starch endosperm ratios (Styer and Cantliffe, 1983A). The result of the leakage of carbohydrates and amino acids could increase pathogenic attack. Soil pathogens grow well in the low soil temperatures that induce chilling injury of corn seeds (Leach, 1974). In most instances pathogens attack the seed before it can germinate successfully.

Dehydration of seeds with high sugar/starch endosperm ratios reduces pericarp and cell membrane integrity when compared to seeds with low sugar/starch endosperm ratios (Wann, 1986). The pericarp of many supersweet corn seed types has large, visible holes which allow increased velocity of water uptake by the imbibing seed (Styer and Cantliffe, 1983A).

This rapid influx of water results in accelerated hydration of the embryo which increases the potential for seed damage and leakage (Styer and Cantliffe, 1983A). Another cause of chilling injury associated with seed drying is loss of membrane integrity. Before drying, cell membranes are oriented into a lamellar configuration known as the phospholipid bilayer. During seed dehydration the phospholipids orient themselves into a hexagonal configuration (Bewley and Black, 1986). During the first few minutes of imbibition when membrane integrity is re-established, solute components can move out of the cells of the seed (Simon, 1974). In the embryo, lack of membrane integrity can allow a rapid influx of water which can damage the embryo cells (Herner, 1986). Low germination temperatures inhibit the rapid re-establishment of the membrane fluid mosaic structure causing the lipid tail portion of the molecules composing the membrane to remain in a less fluid, gel-like phase. This gel-like phase increases the potential for chilling injury by inhibiting the reorganization of the fluid mosaic configuration of the membrane (Bewley and Black, 1986).

Factors Influencing Seed Vigor

The spring planting season for supersweet corn seed in the Midwest is very brief. The minimum soil temperatures for germination of sweet corn seed are 10 to 12 C (Coons, 1986).

The optimum soil temperatures for sweet corn seed germination are 16 to 35 C (Coons, 1986). Average soil temperature at depths of 10 cm in Illinois for the spring planting season is 15 C (Ware and McCollum, 1980)

Later plantings, when soil temperatures are higher, exhibit reduced yields (Kaukis and Davis, 1986) and growers miss the profitable early market prices. The possibility of adverse weather conditions and various plant diseases or pests, plantings after May often result in reduced yields. During the brief spring planting season, sweet corn growers and researchers are challenged with problems of germinating and growing a warm season crop in a temperate climate.

Differences in sweet corn seed vigor, as measured by germination percentage and radicle length, are reported between traditional and supersweet genotypes (Wann, 1986). Traditional sweet corn had a significantly higher vigor index (obtained by multiplying % germination by radicle length) than supersweet corn (Wann, 1986). Differences in seed vigor also were seen between different supersweet corn cultivars when planted in cold and warm soils (Tsengwa, 1991).

At low soil temperatures during spring planting seasons, soil pathogens become aggressive. Several species of fungi (Pythium, Gibberella, Diplodia, Fusarium) parasitize sweet corn seeds (Kaukis and Davis, 1986). Metabolite leakage from the supersweet corn kernel during imbibition allows soluble sugars to move from the kernel's endosperm into the soil. The

increased sugar concentration in the soil immediately surrounding the seed encourages fungi growth leading to seed decay (Pieczarka and Wolf, 1978). Therefore, supersweet corn cultivars with a relatively lower sugar content or less metabolite leakage could show a lower incidence of pre-emergence mortality due to seed rot by pathogens.

Electrical Conductivity of Sweet Corn Seed Leachate

During imbibition water soluble components of sweet corn seeds have the potential to leak from seeds into the surrounding soil. Examples of water soluble seed components that leak from imbibing seeds are: sugars, organic acids, gibberellic acid, soluble proteins, amino acids, phenolics, phosphates, succinate, enzymes, and ions (Herner, 1986). Water in which a corn seed is imbibed contains leached ions that affect electrical conductivity of the imbibitional water. A conductivity bridge indicates the concentration of leached ions. Conductivity tests (expressed in umhos) are good indicators of the amount of ion leakage from a seed. However, conductivity tests do not indicate what specific ions have leaked from seeds.

Leaching of electrolytes from seed during the early stages of germination is associated with poor seed quality and low field emergence percentages of supersweet corn (Waters and

Blanchette, 1983). Conductivity data is negatively correlated with germination and field emergence. This strong correlation allows conductivity data to be used as a quick and useful tool to aid in the prediction of supersweet corn field emergence (Tracy and Juvik, 1988).

Imbibition of sweet corn seeds at low temperatures causes increased and sustained leakage of metabolites from the seed (Simon, 1974). During the first few minutes of imbibition of dry seeds, water rapidly moves into the seed as metabolites leak out. This rapid exchange process decreases as imbibition proceeds (Simon and Mills, 1983). As previously mentioned, low temperature hinders the restablishment of cellular lipid membranes which function to regulate movement into and from cells of seeds.

Not only does temperature affect leakage, cultivars with different endosperm genotypes also differ relative to seed leakage as measured by conductivity (Wann, 1986). Non-traditional sweet corn genotypes consistently show higher seed leachate conductivity than traditional endosperm genotypes (Schmidt and Tracy, 1989).

Components of Supersweet Corn Seed

A longitudinal section of a corn kernel (Fig.1) shows the main regions involved during imbibition. The pericarp, or seed coat, is the kernel's prominent outermost structure and is derived from the ovary wall. Sweet corn tenderness is determined by the thickness of the pericarp present or the thickness of the cell walls that compose this layer (Tracy and Galinat, 1987). Pericarp tenderness is an important factor in determining quality of canned sweet corn. According to Bewley and Black, 1986, seeds of some species germinate very poorly and show reduced growth and vigor when imbibed without the pericarp. Inspection of the pericarp of 'Illini Gold' and 'Honey'n'Pearl' supersweet corn kernels, using maginifications of 3 to 10x revealed holes in the pericarp. Areas in which the pericarp separated from the aleurone layer were also observed. Kernels with damaged pericarps also show a high level of seed leachate electrical conductivity (Schmidt and Tracy, 1989).

The aleurone layer is composed of proteins and lipids. Enzymes, such as proteases and alpha amylase, are synthesized in the aleurone layer and act to catalyze some of the reactions associated with germination (i.e. hydrolysis of storage proteins to amino acids and mobilization of endosperm reserves; Bewley and Black, 1986).

The endosperm is a triploid nutritive tissue used by the embryo at germination. Depending upon kernel genotype, endosperm is composed of insoluble starches, soluble sugars,

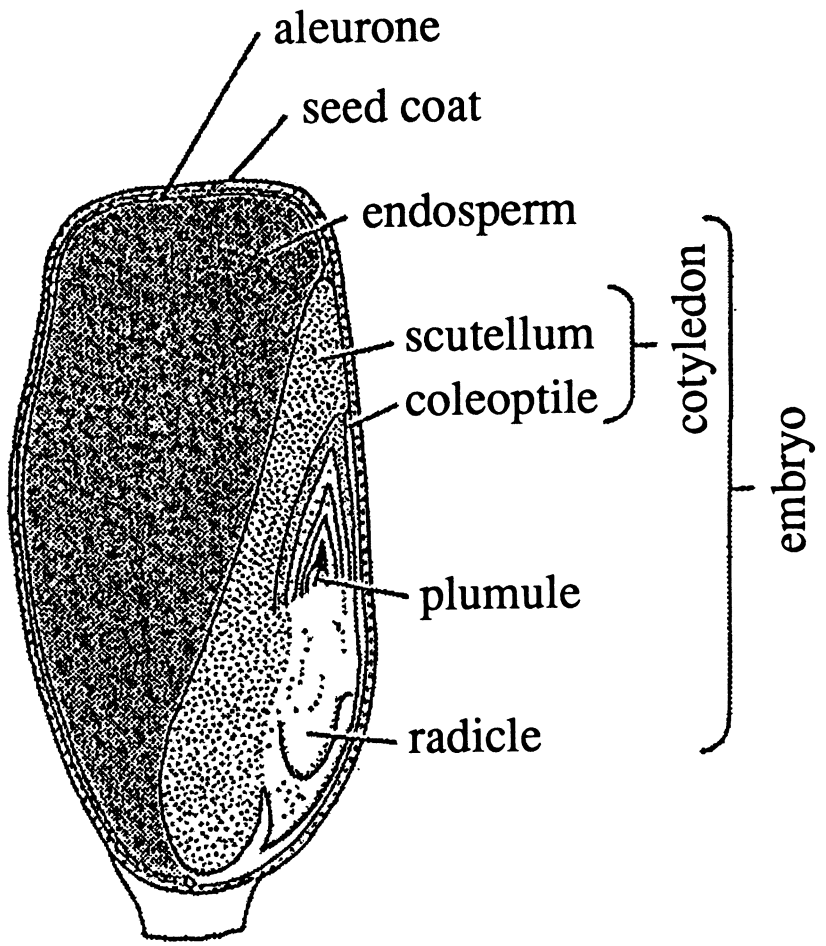


Figure 1. Longitudinal section of a corn kernal.
(Adapted from Fig. 12-28, Wilson, Loomis and Steeves, 1971).

and soluble polysaccharides (Kaukis and Davis, 1986). Endosperm composition can influence the drying process of corn kernels. Kernels with a relatively higher sugar/starch ratio dry into a more wrinkled seed than kernels with a lower endosperm sugar/starch ratio. The relatively high sugar composition of the endosperm gives the supersweet corn kernel its desirable sweet taste.

The embryo is a dormant miniature plant which consists of an embryonic shoot, root, and primordial leaves (Salisbury and Ross, 1992). In corn, enzymes responsible for mobilization of seed reserves are directed by hormonal factors (i.e. gibberellins) produced by the germinating embryo (Bewley and Black, 1986).

During imbibition, any seed component that is soluble in water has the potential to leach from the seed and may result in pre-emergence seedling mortality. A number of studies have been conducted to determine factors that contribute to metabolite leakage from supersweet corn seed and to determine which seed components leach from imbibing seeds. Since there are no generalized tests to identify leachate components, leachate was analyzed for suspected components. Specific seed components analyzed in this study were chosen due to their importance during seed imbibition and germination.

Germination, Emergence, and Pre-emergence Mortality

Seed germination begins with imbibition and ends with radicle protrusion from the kernel. Upon imbibition, the shrunken, dehydrated cell walls of the embryo expand. These changes in the cell walls occur in nonliving, dormant, or viable-nondormant seeds (Bewley and Black, 1986). Completion of germination is determined by radicle expansion and penetration of the outermost coverings of the seed. Penetration requires properly functioning cell membranes and coordinated physiological events including synthesis of appropriate enzymes and mobilization of storage reserves (i.e. carbohydrates).

In supersweet corn kernels, storage reserves are water insoluble starch, water soluble starch, and water soluble sugars. Endosperm of supersweet sh-2 genotypes has a higher sugar/starch ratio relative to endosperm of traditional su genotypes (Styer and Cantliffe, 1983A). High rates of pre-emergence mortality of supersweet corn kernels may be explained by considering that: 1) kernel endosperm is the source of energy for seedling emergence; and 2) supersweet corn kernel endosperm sugars are soluble in water and may be lost from the kernel during imbibition.

Before endosperm sugars are mobilized for energy for radicle emergence, amino acids are required for synthesis of the enzymes that catalyze the usage of the endosperm sugars. The high rate

of pre-emergence mortality of supersweet corn kernels may be explained by considering that: 1) seed protein reserves are hydrolized to produce amino acids for enzyme synthesis to promote metabolic processes necessary for seedling emergence; and 2) some of the supersweet corn kernel proteins are soluble in water and may be lost from the kernel during imbibition (Wann, 1986).

Calcium, Potassium, and Cell Membrane Activity

Different seed components are lost from within cells of supersweet corn kernels during imbibition. Therefore, it is reasonable to assume that selectively permeable cell membranes that retain cell solutes have lost their regulatory ability. Cell membranes in dry kernels require about 25% hydration to retain their functional phospholipid bilayer configuration (Bewley and Black, 1986). In addition to membrane hydration, cation interactions play a vital role in maintenance of selective permeability of membranes (Ferguson and Drobak, 1988). Ferguson and Drobak (1988) suggested that extracellular pools of calcium ions regulate both intercellular calcium pools and ionic interactions of plasma membrane structure and function. Calcium binding to membrane phospholipids and proteins affects phase transitions and fluidity of membranes (Ferguson and Drobak, 1988). Both phase transition and membrane fluidity are

are influenced by temperature and are very critical to successful germination and emergence of corn seeds.

Many cell functions require an energy source to transport anions, cations, or neutral molecules across cell membranes. In 1978, Peter Mitchell won the Nobel prize in chemistry for his work showing proton pumps provide cells with two usable sources of energy: the pH gradient and the electropotential gradient (Salisbury and Ross, 1992). In addition to maintaining ion gradients for membrane functions, potassium: 1) activates many enzymes essential for cellular respiration, and 2) contributes to osmotic potential which influences cell turgor pressure (Salisbury and Ross, 1992). In an imbibing seed, enzymatic activation for metabolism and turgor pressure regulation for the hydrating embryo are critical to successful germination and emergence of corn seed.

Leakage of potassium ions during seed development (Styer and Cantliffe, 1983A) and leakage of soluble proteins (Wann, 1986) varies between genotypes. Leakage of potassium ions during seed development was similar to that of total electrolyte conductivity (Styer and Cantliffe, 1983A). Leakage of soluble protein (expressed in milligrams per seed) during imbibition was relatively higher from seeds with high sugar endosperm content (Wann, 1986). When soluble protein leakage was expressed on a per gram seed basis, differences between genotypes were not significant. From this data, Wann (1986) deduced that protein leakage was not directly related to the amount of soluble

protein present in seeds, but could be a response to defective seed membranes. These differences between genotypes could also be a result of more rapid solubilization during early imbibition in high-sugar genotypes than in su genotypes. Leaching of soluble proteins during early imbibition would reduce the availability of necessary amimo acids hydrolyzed from those proteins.

CHAPTER 3

MATERIALS AND METHODS

Untreated supersweet corn seed (Zea mays L. var. rugosa) from Illinois Foundation Seed, Inc. (Champaign, IL) was used in this study. Two cultivars, 'Illini Gold' and 'Honey'n'Pearl', were chosen based on their susceptibility to chilling injury during germination. Chilling injury susceptibility was determined by field emergence in low temperature plantings of five supersweet corn cultivars in 1991 by Nombasa Tsengwa on University of Illinois farm plots (Appendix A). From the five cultivars observed, a tolerant cultivar, 'Illini Gold', and a sensitive cultivar, 'Honey'n'Pearl', were chosen. Seeds were stored at 40 C in paper packets and placed in a 9 x 13 covered plastic container with approximately 2 centimeters of desiccant (Drierite) lining the bottom to control humidity.

Seeds were imbibed in the dark at 10.0 $\pm$ 1.0, 14.9 $\pm$ 0.7, and 19.8 $\pm$ 0.8 C in a growth chamber (Sherer-Gillett, Co., Model CEL 25-7, Marshall, MI). Prior to imbibition, the temperature of seeds and distilled water were allowed to equilibrate by setting in the growth chamber at the prospective imbibitional temperature. Temperature in the growth chamber was determined and monitored daily using a mercury thermometer because it best reflected temperatures

realized by seeds surrounded by water which tends to resist quick and minute temperature changes.

Germination

Preliminary studies were performed to determine number of seeds, amount of deionized water, and germination substrate for the germination tests (Appendix B). Based on these studies, seeds were evenly placed on 3 sheets of filter paper (Whatman no. 1) and moistened with 10 ml of distilled water in glass petri dishes (9 cm x 1.5 cm). Three replications per cultivar and temperature with 20 seeds/replication were imbibed. Dishes were kept in a covered plastic container to minimize water evaporation during germination. No additional water was added.

Seeds were considered germinated when the radicle protruded 1 cm from the seed surface. Dishes were checked every 24 hours for 14 days. After the number of germinated seeds was recorded, germinated seeds were removed from petri dishes. Fungal and bacterial growth were noted in each dish. After 14 days, percent germination and days to 25% germination were calculated.

Ion Leakage

Preliminary studies were performed to determine at what point during imbibition to conduct tests (Appendix B). Based on the studies, the equilibration time was established at 3 hours. For each cultivar and temperature, ten replications of 2 seeds/10 ml of distilled water were imbibed in plastic test tube caps (38 mm x 18 mm).

After 3 hours at the respective imbibitional temperature, electrical conductivity of the 10 ml of imbibitional water, which will henceforth be referred to as seed leachate, was recorded for each replication. Seeds were not removed from their imbibitional water for this reading. Electrical conductivity (recorded in umhos with the meter set at 85Hz) was measured with a Beckman Altrex Conductivity Bridge, model RC-16C, with a Beckman Conductivity Cell, model G01. Seeds and seed leachate of each replication were then uniformly ground using a Brinkman Polytron homogenizer PT 3000 with a Brinkman 89/Polytron probe, 12mm, PT-DA 3012/2. The resulting seed solutions were filtered through Calbiochem Miracloth filtration material, and the electrical conductivity of the ground seed filtrate was measured.

Percent ion leakage was calculated as the pre-grind electrical conductivity divided by the post-grind electrical conductivity multiplied by 100.

Sugars

For each cultivar and temperature, ten replications of 2 seeds/10 ml distilled water were imbibed for 3 hours as previously explained. After imbibition, the seeds were immediately removed from each plastic cap and discarded. In order to correlate sugar content of seed leachate (ug/seed/ml) with electrical conductivity of the same seed leachate (umhos/seed/ml) each replication was labeled and measured for the two respective components. Electrical conductivity was measured as previously explained.

A colorimetric anthrone method (Umbriet, Burris, and Staufer, 1959) was used to analyze seed for sugar content. The leachate was filtered using Calbiochem Miracloth before continuing with the sugar analysis. This filtration eliminated the possibility of debris from seed surfaces interfering with the Spectrophotometer-20 reading. Anthrone reagent (100 mg anthrone per 50 ml concentrated sulfuric acid; Fisher Scientific Company, New Jersey) was mixed prior to each test, stored at room temperature in a dark glass bottle, and discarded if not used within 24 hours after mixing. Anthrone reagent produces blue-colored complexes with most soluble sugars and polysaccharides (after hydrolysis). Using a 1 ml pipette, 0.5 ml of each seed leachate replication was placed into appropriately labeled test tubes. The contents of each test tube were then diluted with 0.5 ml distilled water.

Using a burette, 3 ml of anthrone reagent was added to the diluted samples in each test tube. Two water-anthrone blanks were prepared using 1 ml of distilled water plus 3 ml of anthrone reagent. The sample test tubes and blanks were placed in a boiling water bath for 10 minutes, removed and quickly cooled to room temperature in an ice bath. The resulting solutions in the test tubes were read using a Spectrophotometer-20 at 620 nm and compared to the lowest water-anthrone blank. A sucrose standard curve was prepared in the same manner using sucrose concentrations of 20, 40, 60, 80, and 100 ug/ml from a stock solution of 10 mg sucrose/100 ml (Appendix C). Sugar was calculated using the standard curve.

Sugar content was calculated as ug sugar/seed/ml, and electrical conductivity was calculated as umhos/seed/ml.

Amino Acids

For each cultivar and temperature, 10 replications of 3 seeds per 5 ml distilled water were imbibed for 3 hours as previously explained. After imbibition, seeds were immediately removed from each plastic cap, and electrical conductivity of seed leachate was recorded. In order to determine the association of amino acids (ug/seed/ml) of seed leachate with electrical conductivity of the same seed leachate

(umhos/seed/ml), each replication was labeled and measured for the two respective components. Electrical conductivity was measured as previously explained.

The colorimetric ninhydrin method of analysis (Moore and Stein, 1954) was used to quantitatively analyze seed leachate for amino acids. The ninhydrin reagent was prepared as follows:

Hydrindantin: One solution was prepared by dissolving 8 g ninhydrin (Aldrich Chemical Company, Milwaukee, WI) into 200 ml distilled water at 90 C. A second solution was prepared by dissolving 8 g ascorbic acid (Fisher Scientific Company, New Jersey) in 40 ml distilled water at 40 C. The solutions were maintained at their respective temperatures only until the compounds were dissolved. No further heating was necessary. The two solutions were then combined in a 500 ml beaker and gently stirred by hand for 5 seconds. Crystallization began within 15 seconds after mixing and was allowed to proceed for 30 minutes. After 30 minutes the beaker was placed in a large bowl of cool tap water to allow the crystallizing compound to cool to room temperature (about 1 hour). The crystallized hydrindantin was filtered through Miracloth, washed well with distilled water, and filtered again with dry Miracloth to reduce moisture content of the crystallized hydrindantin. The moist hydrindantin crystals were placed into a dark glass bottle and dried in the dark in a drying oven at 25 C with a blower for approximately 24 hours. The hydrindantin powder was stored in a dark glass bottle with the lid tightened securely.

4N Sodium Acetate Buffer (pH 5.5): A solution was prepared by adding 136 g NaOAc 3H 0 (Fisher Scientific Company, New Jersey)to 100 ml warm distilled water (about 45-50 C). The water temperature was maintained until all the sodium acetate was dissolved. After the solution was cooled to room temperature, 25 ml of glacial acetic acid (Fisher Scientific Company, New Jersey) was added. The resulting sodium acetate buffer solution was diluted to a volume of 250 ml with distilled water and the pH was tested using a Corning pH meter model 10. If final adjustment of the pH was necessary, 5 g of NaOH corresponded to 0.04 pH units. The buffer was stored in a plastic bottle in a refridgerator.

Ninhydrin Reagent: Ninhydrin reagent was made just prior to use to avoid storage procedure and the unused portion was discarded. Into 41.6 ml of Cellosolve (Sargent-Welch Scientific Company, Skokie, Illinois), 1.11 g ninhydrin and 0.166 g hydrindantin were dissolved. At room temperature, this solution was gently hand-stirred with a glass rod. No bubbles resulting from agitation were allowed in the solution. After complete dissolution, 13.83 ml of sodium acetate buffer was added and the solution gently stirred for 5 seconds. The resulting brown reagent was immediately transferred to a dark glass bottle and placed away from light until used.

Standard Curve: A leucine standard curve was prepared from a stock solution of 200 ug/ml. Leucine (Calbiochem, San Diego,

California) was chosen because it is the predominant amino acid found in plants and is less likely to be broken down during plant metabolic processes. The stock solution was diluted to yield a concentration series of 100, 50, 37, 25, 15, and 10 ug/ml. These concentrations were analyzed according to the following procedure and a standard curve plotted (Appendix C).

Basic Procedure: To determine amino acids present in seed leachate and in the concentration series of the leucine standard curve, a 2 ml volume of each sample solution (i.e. seed leachate or standard curve solutions) was pipetted into appropriately labeled test tubes containing 1 ml of ninhydrin reagent. Two water-ninhydrin blanks were prepared using 2 ml of distilled water and 1 ml ninhydrin reagent. Capped tubes were vigorously shaken by hand for 8-10 seconds and heated for 15 minutes in a gently boiling water bath. After removal from the water bath, 6 ml of a 50:50 ethanol-water solution was added to each test tube and the test tubes allowed to cool to below 30 C. Prior to Spectrophotometer-20 readings at 580 nm, each test tube was thoroughly shaken (about 20 seconds) to reduce any residual hydrindantin. Sample test tubes were compared to the lowest water-ninhydrin blank. Absorbance readings were recorded for the seed leachate and the leucine standard curve. Amino acids were calculated using the standard curve.

Calcium and Potassium Ions

For each cultivar, 3 replications of 40 seeds per 20 ml distilled water were imbibed for 3 hours as previously explained. Plastic bottles (50 ml) were used instead of the 12 mm plastic test tube caps previously described. After imbibition, seeds were immediately removed from each plastic bottle, and the electrical conductivity of seed leachate was recorded. In order to correlate three parameters (i.e. calcium ion content in ug/seed/ml, potassium ion content in ug/seed/ml, and electrical conductivity in umhos/seed/ml) of the same seed leachate, each replication was labeled and measured for the three respective components. Electrical conductivity was measured as previously explained. Calcium and potassium ions in seed leachate were determined by atomic emission spectroscopy (Chapman and Pratt, 1961).

Atomic emission was measured using a Perkin-Elmer Atomic Absorption Spectrometer (model 2380) using an air-acetylene flame (2400 C). Samples were aspirated at least 30 seconds before readings were taken. Each reading was the average of 10 separate emission determinations at each respective wavelength. Calcium emissions were read at a wavelength of approximately 544.0 nm (the wavelength maxima for CaO) and potassium emissions at approximately 766.4 nm. Fine adjustments of the wavelength setting for each analyte were made as needed.

A calcium standard curve (calcium nitrate; Fisher Scientific

Company, New Jersey) was prepared from a stock solution of 200 ug/ml of calcium. The stock solution was diluted to yield a concentration series of 200, 100, 50, 25, and 10 ug/ml of calcium (Appendix C). A potassium standard curve (potassium chloride; Fisher Scientific Company, New Jersey) was prepared from a stock solution of 240 ug/ml of potassium. The stock solution was diluted to yield a concentration series of 180, 120, 60, 30, and 15 ug/ml of potassium (Appendix C). For construction of standard curves, relative emission intensity was plotted against analyte concentration (ug/ml). The standard curves were linear. Potassium and calcium were calculated using standard curves.

Statistical Analyses

All parameters were analyzed using a two-way analysis of variance. The two factors involved in each ANOVA were cultivar and temperature. When a two-way analysis of variance revealed an interaction between factors, data for individual cultivars and individual temperatures were considered separately by one-way analyses of variance. The experimental design was completely randomized. Means were separated by Duncan's multiple range test at significance level of $P = 0.05$. The CoStat computer program was used for all statistical analyses.

CHAPTER 4

RESULTS

The two-way analysis of variance for % germination of cultivar by temperature revealed no significant interaction of factors, but cultivar and temperature had significant effects (Appendix D). Percent germination was significantly higher for 'Illini Gold', a tolerant cultivar, than for 'Honey'n'Pearl', a sensitive cultivar (Table 1). For both cultivars, percent germination at 10 C was significantly lower than at 15 and 20 C.

The two-way analysis of variance for days to 25% germination revealed that all factors and all interactions of factors were significant (Appendix D). Thus, data for individual cultivars and individual temperatures were considered separately by one-way analyses of variance. At all temperatures, days to 25% germination were higher for 'Honey'n'Pearl' than for 'Illini Gold' (Table 2). For 'Illini Gold', days to 25% germination was significantly different at all temperatures. For 'Honey'n'Pearl', days to 25% germination were higher at 10 C than at 15 or 20 C.

The two-way analysis of variance for percent ion leakage of cultivar by temperature revealed no significant interaction of factors, but cultivar had a significant effect (Appendix D). Percent ion leakage was significantly higher for 'Honey'n'Pearl' than for 'Illini Gold' (Table 3). For both cultivars, percent ion leakage was significantly higher at 20 C than at 10 C, but neither 10 nor 20 C were significantly different from 15 C.

Table 1. Percent germination after 14 days at 10, 15, and 20°C for two cultivars of sweet corn.

	Germination (%)
Cultivar	
Illini Gold	93 ± 9 a[z]
Honey'n'Pearl	47 ± 14 b
Temperature (°C)	
10	56 ± 29 b
15	74 ± 27 a
20	79 ± 21 a

[z]means are lumped because of no significant interaction; mean separation for lumped means within cultivar or temperature based on Duncan's multiple range at the P=0.05.

Table 2. Days to 25% germination at 10, 15, and 20°C for two cultivars of sweet corn.

Cultivar	Temperature (°C)		
	10	15	20
Illini Gold	6.7 ± 0.3 bw[z,y]	2.7 ± 0.3 bx	1.7 ± 0.3 by
Honey 'n' Pearl	11.5 ± 2.3 aw	4.7 ± 0.3 ax	2.7 ± 0.3 ax

[z]ab to separate means within a column based on Duncan's multiple range at P=0.05.
[y]wxy to separate means within a row based on Duncan's multiple range at P=0.05.

Table 3. Electrical conductivity (%) of seed leachate after 3 hours imbibition at 10, 15, and 20°C for two cultivars of sweet corn.

	Electrical conductivity (%)	
Cultivar		
Illini Gold	6.0 ± 1.5	b[z]
Honey'n'Pearl	13.3 ± 4.0	a
Temperature (°C)		
10	8.6 ± 4.3	b
15	9.5 ± 4.5	ab
20	10.8 ± 5.4	a

[z]means are lumped because of no significant interaction; mean separation for lumped means within cultivar or temperature based on Duncan's multiple range at the P=0.05.

The two-way analyses of variance for potassium and electrical conductivity in seed leachate of cultivar by temperature revealed no significant interaction between factors, but cultivar and temperature had significant effects (Appendix D). Potassium in seed leachate was significantly higher for 'Honey'n'Pearl' than for 'Illini Gold' (Table 4). For both cultivars, potassium in seed leachate was significantly higher at 10 and 20 C than at 15 C. For electrical conductivity associated with analysis of potassium in seed leachate, 'Honey'n'Pearl' had significantly higher electrical conductivity in seed leachate than 'Illini Gold' for all temperatures (Table 4). For both cultivars, electrical conductivity of seed leachate was significantly higher at 15 and 20 C than at 10 C.

The two-way analyses of variance for sugars and electrical conductivity in seed leachate of cultivar by temperature revealed no significant interaction of factors (Appendix D). For sugars in seed leachate, cultivar had a significant effect. For electrical conductivity of the same seed leachate, cultivar and temperature had significant effects. Sugars in seed leachate were significantly higher for 'Honey'n'Pearl' than for 'Illini Gold' (Table 5). No significant temperature differences for sugars in seed leachate were found. Electrical conductivity associated with analysis of sugar in seed leachate was

Table 4. Potassium (ug/seed/ml) and electrical conductivity (umhos/seed/ml) of seed leachate after 3 hours imbibition at 10, 15, and 20°C for two cultivars of sweet corn.

	Potassium (ug/seed/ml)	Electrical conductivity (umhos/seed/ml)
Cultivar		
Illini Gold	0.106 ± 0.015 b[z,y]	3.90 ± 0.42 b
Honey'n'Pearl	0.168 ± 0.019 a	8.18 ± 0.87 a
Temperature (°C)		
10	0.151 ± 0.041 a	5.43 ± 2.01 b
15	0.117 ± 0.032 b	6.15 ± 2.72 a
20	0.143 ± 0.031 a	6.55 ± 2.41 a

[z]means are lumped because of no significant interaction between cultivar and temperature.
[y]mean separation of lumped means within a column for cultivar or temperature based on Duncan's multiple range at the P=0.05.

Table 5. Sugar (ug/seed/ml) and electrical conductivity (umhos/seed/ml) of seed leachate after 3 hours imbibition at 10, 15, and 20°C for two cultivars of sweet corn.

	Sugar (ug/seed/ml)	Electrical conductivity (umhos/seed/ml)
Cultivar		
Illini Gold	1.79 ± 0.58 b[z,y]	14.93 ± 3.60 b
Honey'n'Pearl	2.96 ± 0.97 a	28.50 ± 9.14 a
Temperature (°C)		
10	2.20 ± 0.80 a	19.08 ± 9.96 b
15	2.41 ± 1.08 a	20.01 ± 8.59 b
20	2.50 ± 1.07 a	26.07 ± 9.44 a

[z]means are lumped because of no significant interaction between cultivar and temperature.
[y]mean separation of lumped means within a column for cultivar or temperature based on Duncan's multiple range at the P=0.05.

significantly higher for 'Honey'n'Pearl' than for 'Illini Gold' (Table 5). For both cultivars, electrical conductivity of seed leachate was significantly higher at 20 C than at 10 and 15 C.

The two-way analysis of variance for amino acids in seed leachate of cultivar by temperature revealed no significant interaction between factors (Appendix D). For amino acids in seed leachate, cultivar had a significant effect. For electrical conductivity of the same seed leachate, cultivar and temperature had significant effects. Amino acids in seed leachate were significantly higher for 'Honey'n'Pearl' than for 'Illini Gold' (Table 6). No significant temperature differences for amino acids in seed leachate were found. For electrical conductivity associated with analysis of amino acids in seed leachate, 'Honey'n'Pearl' had significantly higher electrical conductivity than 'Illini Gold' (Table 6). For both cultivars, electrical conductivity of seed leachate was significantly higher at 15 and 20 C than at 10 C.

A two-way analysis of variance for calcium in seed leachate could not be calculated because of lack of data due to low amounts of calcium in seed leachate as determined by atomic emission spectroscopy.

Table 6. Amino acids (ug/seed/ml) and electrical conductivity (umhos/seed/ml) of seed leachate after 3 hours imbibition at 10, 15, and 20°C for two cultivars of sweet corn.

	Amino acids (ug/seed/ml)	Electrical conductivity (umhos/seed/ml)
Cultivar		
Illini Gold	1.32 ± 0.39 b[z,y]	70.80 ± 21.73 b
Honey'n'Pearl	3.62 ± 0.75 a	137.31 ± 18.47 a
Temperature (°C)		
10	2.36 ± 1.34 a	92.77 ± 35.03 b
15	2.46 ± 1.27 a	106.57 ± 38.09 a
20	2.59 ± 1.36 a	112.84 ± 42.83 a

[z]means are lumped because of no significant interaction between cultivar and temperature.
[y]mean separation of lumped means within a column for cultivar or temperature are based on Duncan's multiple range at the P=0.05.

CHAPTER 5

DISCUSSION

Researchers seek quick and simple laboratory procedures to indicate field performance of supersweet corn seeds. During imbibition, any seed component that is soluble in water has the potential to leach from seeds. This loss may result in pre-emergence seed mortality. A conductivity bridge measures electrical conductivity of a solution and is an indication of ion concentration. Electrical conductivity of seed leachate, as a reliable indicator of field performance and seed quality, was inversely related to cultivar germination percentage. 'Illini Gold' had higher germination percentages and lower electrical conductivity readings at all temperatures than did 'Honey'n'Pearl'.

Temperature affects both physical and chemical processes of imbibition and germination. Electrical conductivity for both cultivars was consistently higher at 20 C than at 10 C which could be due to physical effects of temperature on speed of reactions. For days to 25% germination, seeds germinated more rapidly at 20 C than at 10 and 15 C which could be due to temperature effects on enzyme activity associated with germination. At 20 C, seeds germinated sooner, germination percentages were higher, but ion leakage also was higher than at 10 C. Although ion leakage potentially reduces seed vigor,

germination at optimum temperatures may allow the supersweet corn seed to overcome problems associated with imbibitional leakage.

Percent ion leakage for 'Honey'n'Pearl' was 2.2 times higher than for 'Illini Gold'. The temperature effect on percent ion leakage was significant. Although seeds of 'Illini Gold' appear larger than seeds of 'Honey'n'Pearl', previous studies concluded that differences in seed size fail to significantly influence ion leakage (Waters and Blanchette, 1983). Other factors causing significant variability between supersweet corn cultivars are seed moisture, imbibitional water, and seed handling. In this study these factors were not considered to cause significant variability between cultivars since storage moisture levels, storage temperature, and imbibitional water were the same for both cultivars prior to all procedures. When a reduction in variation of environmental factors results in significant differences between cultivars, then the possibility of genetic variation between cultivars should be considered.

Supersweet corn genotypes could affect percent ion leakage in the following manner: 1) mutant genes at the su locus and gene modifiers at other loci can determine carbohydrate composition of endosperm; 2) endosperm carbohydrate composition affects rate of preharvest seed dehydration and rate of imbibitional hydration; and 3) rates of preharvest seed dehydration and imbibitional hydration influence regulatory

structures (i.e. pericarp and cell membranes) that control imbibitional leakage. Therefore, differences in percent ion leakage between 'Illini Gold' and 'Honey'n'Pearl' could be due to endosperm genotype. Since both cultivars incorporate the sh-2 allele, further study might identify the genes affecting endosperm composition at other loci or determine if gene modifiers (i.e. se) are present.

In addition to electrical conductivity, seed leachate was analyzed for calcium and potassium. Calcium in seed leachate was lower than 0.001 ug/seed/ml for all tests and could not be measured accurately using atomic emission spectroscopy. According to Bewley and Black (1986), calcium in seed may be found as an insoluble salt. Calcium salts, along with potassium and magnesium salts, mix insolubly with phytic acid to form phytin. Phytin is found only in globoids of protein bodies which are species specific for location in seeds. In maize and other cereal grains, the largest phytin storage is within the globoids of aleurone grains. No phytin is associated with protein bodies of starchy endosperm, which potentially leak from imbibing seeds (Bewley and Black, 1986). Calcium associated with cell membranes is bound to outer membrane surfaces and, when associated with cell walls is found as calcium pectate. The extent of insolubility of calcium in imbibing seeds may explain the low amount of calcium in seed leachate.

Potassium in seed leachate followed patterns similar to those of electrical conductivity for temperature. This similarity in pattern was not seen for temperature where it was lower at 15 C than at 10 and 20 C. Potassium in seed leachate was measured during three separate sessions. The lack of pattern similarity for temperature could be due to variations in required setup adjustments on the atomic emission spectrometer prior to each session.

Potassium in seed leachate could be affected to a greater extent by degree of membrane hydration than by temperature effects on membrane phase. As previously discussed, low imbibitional temperatures inhibit reorganization of lipid membranes allowing potassium and other electrolytes to leach from seeds. The data reported in this project does not show that leakage of potassium was higher at 10 C than at 15 or 20 C. In this study, the inverse was true. According to Bewley and Black, 1986, about 25 % hydration is required for maintenance of functional membrane configuration. The 5-hour electrical conductivity curve (Appendix B) measured at 20 C indicates equilibration at approximately one hour. Equilibration at one hour could be concurrent with the re-establishment of functional membrane configuration. In order to test if temperature or cultivar has an effect on membrane configuration, electrical conductivity could be measured at 30-minute intervals for 5 hours at 10, 15, and 20 C. Comparison of such curves could

provide insight about factors influencing membrane reorganization in seeds with different endosperm composition.

Sugars in seed leachate for all temperatures were 1.6 times higher for 'Honey'n'Pearl' than for 'Illini Gold'. No significant temperature differences for sugars in seed leachate were observed. 'Honey'n'Pearl' had lower germination percentages and slower germination rates than 'Illini Gold'. Sugar data indicate that more sugar leaks from seeds of 'Honey'n'Pearl' than from 'Illini Gold'. Sugar in soils surrounding seeds encourages pathogen growth. During germination tests, hyphal growth was more abundant around seeds of 'Honey'n'Pearl' than around seeds of 'Illini Gold'. Also noted was a greater abundance of hyphal growth around seeds closely spaced compared to seeds spaced further apart in petri dishes during germination tests. Also, less hyphal growth was observed around seeds germinated at 20 C than at 10 and 15 C. These data and personal observations could be explained by investigating effects of: 1) leachate sugar concentration on hyphal growth; 2) temperatures on hyphal growth; 3) hyphal growth on seed germination; or 4) seed germination on hyphal growth.

Amino acids in seed leachate followed patterns similar to sugar and electrical conductivity. Amino acids in seed leachate were 2.7 times higher for 'Honey'n'Pearl' than for 'Illini Gold', with no significant temperature differences. Hydrolysis of kernel starch reserves for use during germination requires

enzymes (i.e. alpha-amylase) to be synthesized from amino acids. In some Zea mays L. var rugosa hybrids and mutants, the lag phase of alpha-amylase production is a function of gibberellic acid and amino acid concentration (Bewley and Black, 1986). When either factor alone or in combination are increased, the result is a shortened lag phase prior to alpha-amylase production. Decreased levels of amino acids resulting from imbibitional leakage may thus delay the initiation of starch hydrolysis. For imbibing supersweet corn seeds, delay of activities associated with germination could result in increased pre-emergence mortality.

APPENDIX A

CULTIVAR SELECTION

To select a tolerant and a sensitive supersweet corn cultivar, the cold field soil emergence data of five cultivars (25 seeds each) at 15, 32, and 49 days after planting were reviewed (Tsengwa, 1991) (Figure A). 'Illini Gold' was chosen as the tolerant cultivar and 'Honey'n'Pearl' as the sensitive cultivar.

Figure A. Number of sweet corn plants emerged from 25 seeds of five different cultivars at 15, 32, and 49 days after planting in cold field soils.

	15	32	49
Illini Gold	9.0 ± 5.3a*	18.0 ± 4.9a	18.0 ± 4.8a
Florida Staysweet	8.0 ± 2.8a	19.0 ± 2.2a	19.0 ± 2.2a
Crisp'n'Sweet	9.0 ± 4.0a	18.0 ± 2.6a	18.0 ± 3.0a
Honey'n'Pearl	1.0 ± 1.0b	3.6 ± 2.0c	3.5 ± 2.4c
How Sweet It Is	1.0 ± 1.2b	10.0 ± 2.2b	9.4 ± 2.5b

*Mean separation of lumped means within a column for all cultivars based on Duncan's multiple range at P=0.05.

APPENDIX B

PRELIMINARY TESTS

Preliminary tests were conducted to determine procedures for growth chamber germination tests and procedures for imbibition time prior to electrical conductivity tests and other leachate analyses.

The first preliminary test (Figure B1) was done to determine the volume of distilled water and type of substrate to germinate seeds in petri dishes. The second preliminary test (Figure B2) was conducted for 54 hours to determine number of seed per sample, volume of distilled water per sample, and imbibition period required for equilibration prior to leachate analyses. The third preliminary test (Figure B3) was conducted for 5 hours with a sample size of two seeds in 10 ml of distilled water.

Preliminary Germination Test

Two replications/cultivar with 20 seeds/replication were imbibed with 5, 10, or 20 ml distilled water at 20 C for seven days. The treatments used were 3 sheets of filter paper or 1 folded paper towel per glass petri dish. Seeds were considered germinated when the radicle protruded 1 cm from the seed surface.

Percent germination was calculated by dividing the number of seeds germinated per cultivar for each water volume and treatment by the total number of seeds per cultivar (= 40), and then multiplying by 100. The average percent germination for

the two cultivars for each water volume and for each germination substrate was calculated.

The volume of 10 ml distilled water was chosen because it resulted in the greatest average germination percentage (98.8%) with the least amount of bacterial and fungal growth. Filter paper was chosen because bacterial and fungal growth were more prevalent on paper towels than on filter paper.

Preliminary Imbibition Test (B2)

Six replications of 1 and 5 seeds of both 'Illini Gold' and 'Honey'n'Pearl' were imbibed in 5 ml distilled water for 54 hours at 20 C. Electrical conductivity readings were recorded every three hours beginning at zero hours. Readings were terminated at 54 hours because of seed component fermentation (as detected by a sour smell) and/or radicle protrusion. Graphs were constructed with time (hrs) plotted against electrical conductivity (umhos).

Figure B2 represents the electrical conductivity of both cultivars over 54 hours using a seed sample size of one and five. The imbibition period prior to a noticeable electrical conductivity plateau was determined to be between 0-5 hours.

A five-hour imbibition test was conducted using a sample

size of two seeds in 10 and 20 ml distilled water for both cultivars at 20 C. Electrical conductivity readings were recorded every 30 minutes beginning with zero hours. A graph was constructed (Figure B3) with time (hr) plotted against electrical conductivity (umhos).

A water volume of 10 ml/rep was chosen to provide ample solution for analyses and correlation of two leachate parameters. A seed sample size of two/rep was chosen to reduce the observed variability between replications consisting of one seed. A sample of 5 seeds/replication was disregarded because of the limited number of untreated seed available from Illinois Foundation Seed, Inc., and the large number of seed needed to measure six parameters at three temperatures.

Figure B1. Percent germination of two supersweet corn cultivars* after 7 days using filter paper or paper towels with 5, 10, or 20 ml distilled water.

		5 ml	10 ml	20 ml
FILTER PAPER	'Florida Staysweet'	15.0	97.5	87.0
	'Illini Gold'	55.0	100.0	95.0
		35.0%	98.8%	91.3%
PAPER TOWEL	'Florida Staysweet'	20.0	57.5	95.0
	'Illini Gold'	45.0	97.5	92.5
		32.5%	77.5%	93.8%

*'Florida Staysweet' was used as the sensitive cultivar.

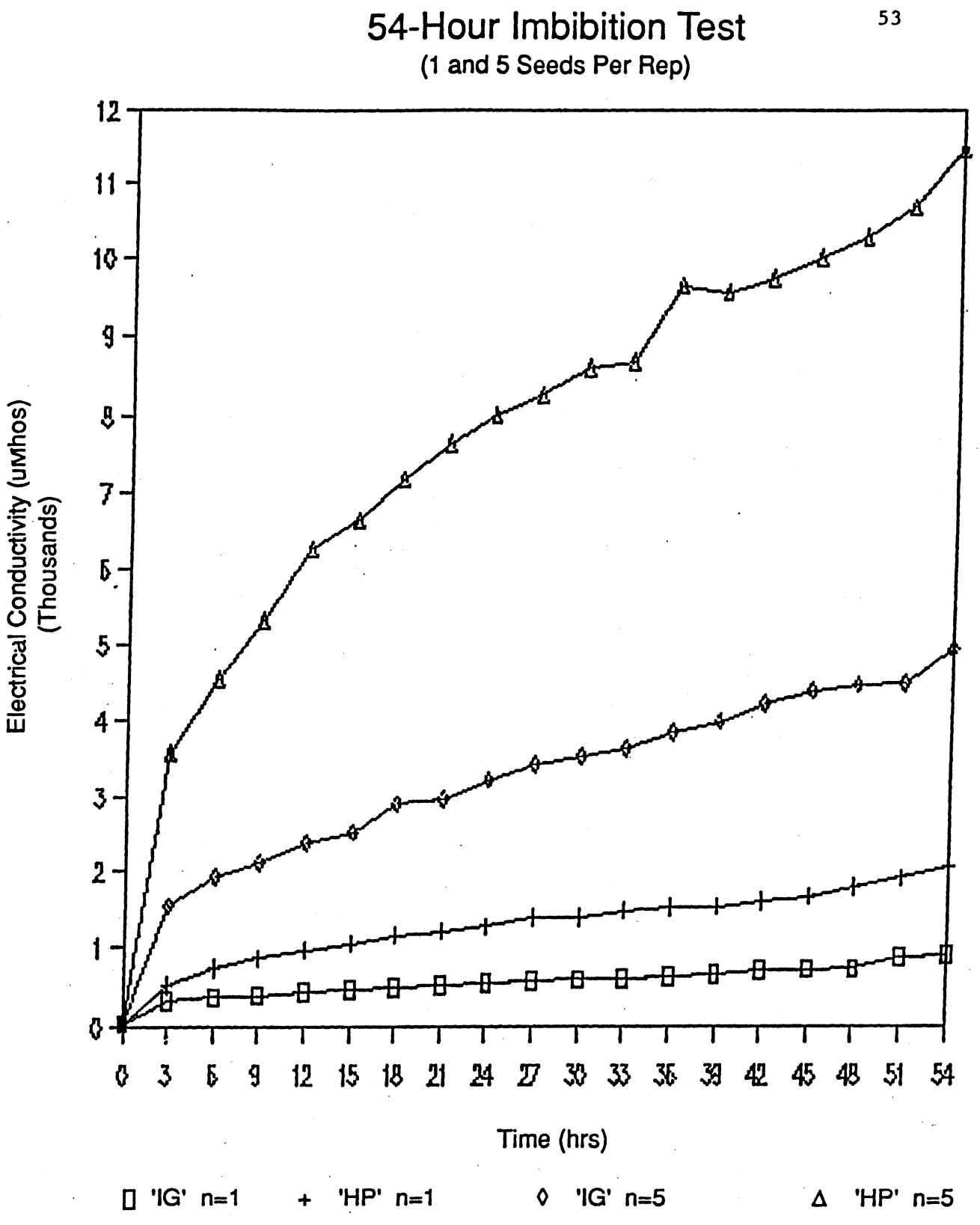


Figure B2. Electrical conductivity (umhos) of seed leachate during imbibition for 54 hours at 20 C for two cultivars of sweet corn and different sample sizes.

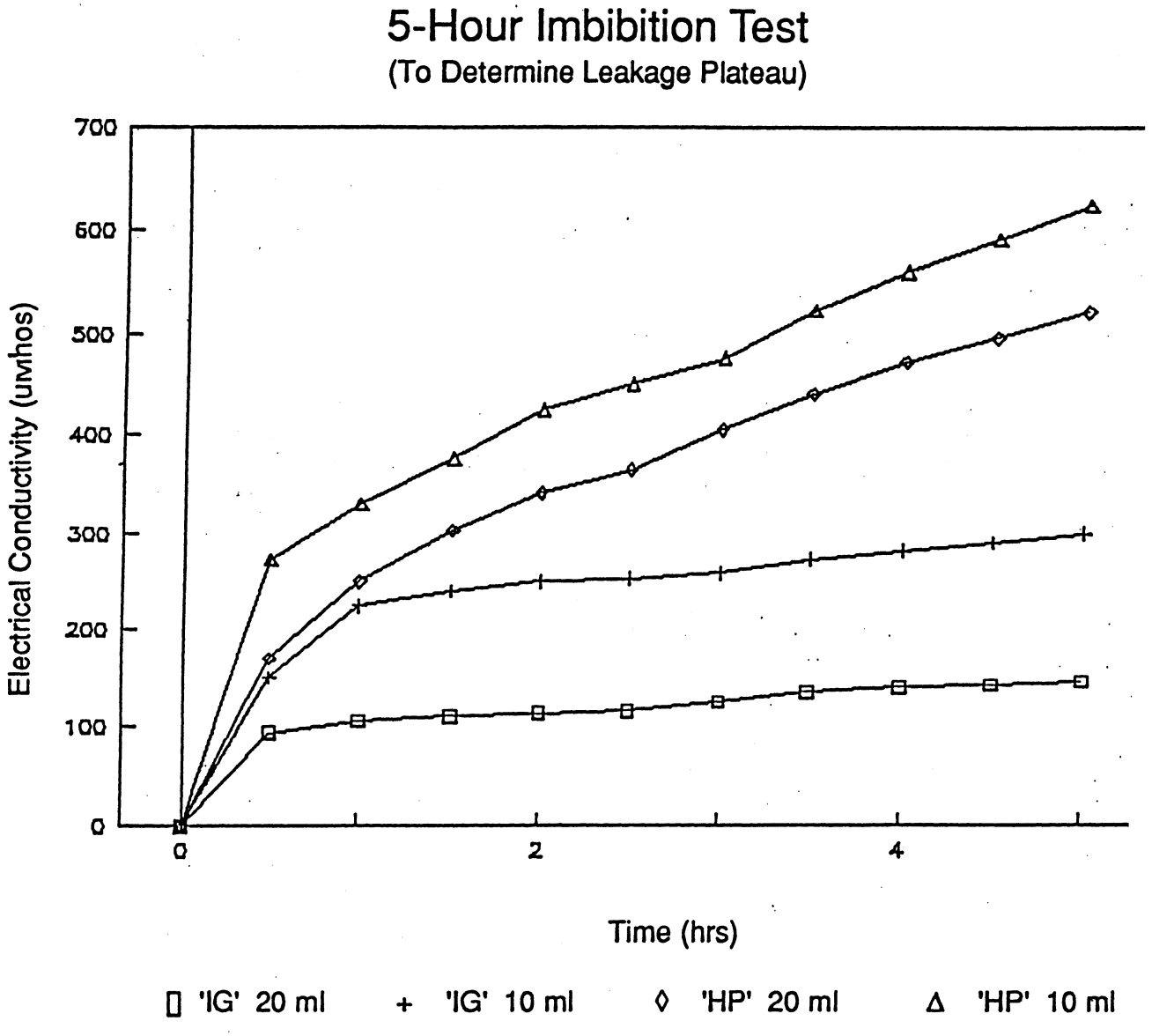


Figure B3. Electrical conductivity (umhos) of seed leachate for 5 hours at 20 C for two cultivars of sweet corn and different volumes of distilled water.

APPENDIX C

STANDARD CURVES

Figure C1. Standard sucrose curve.

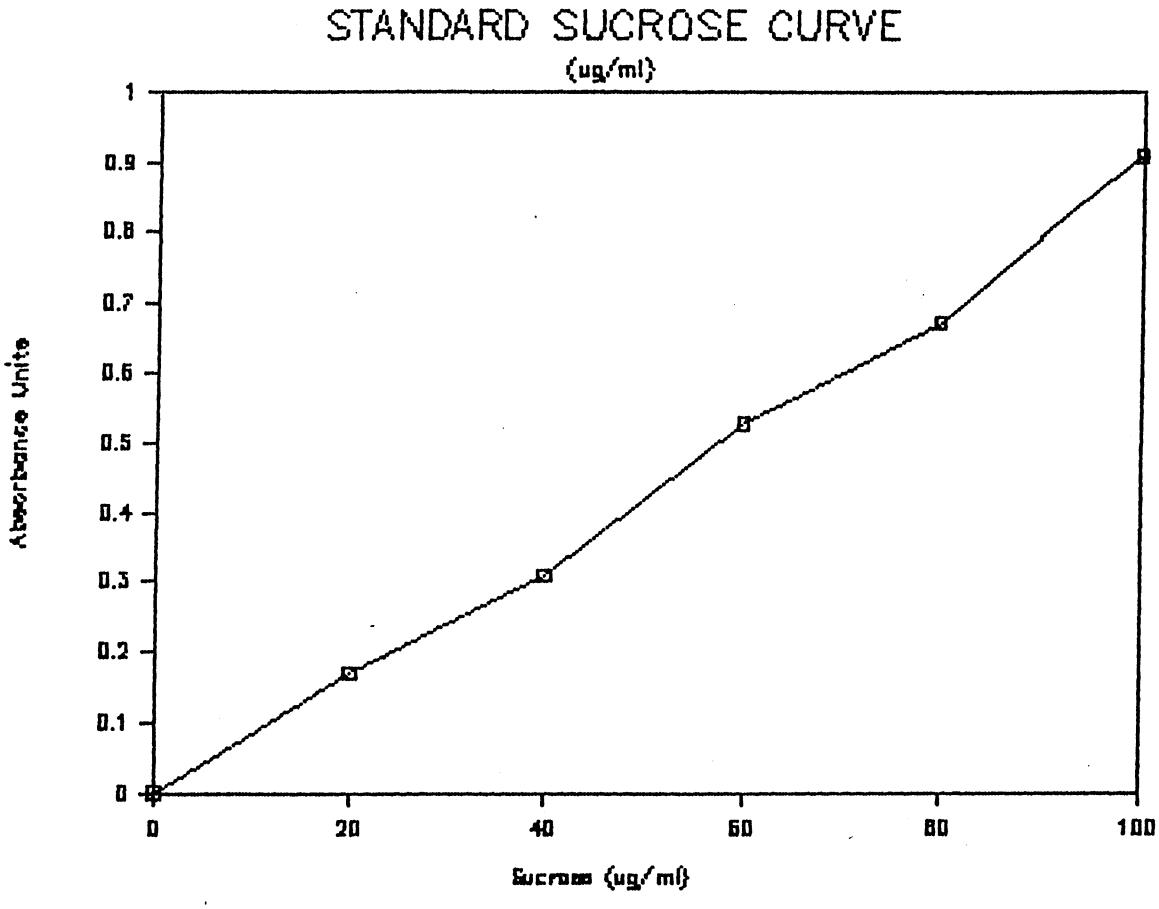


Figure C2. Standard leucine curve.

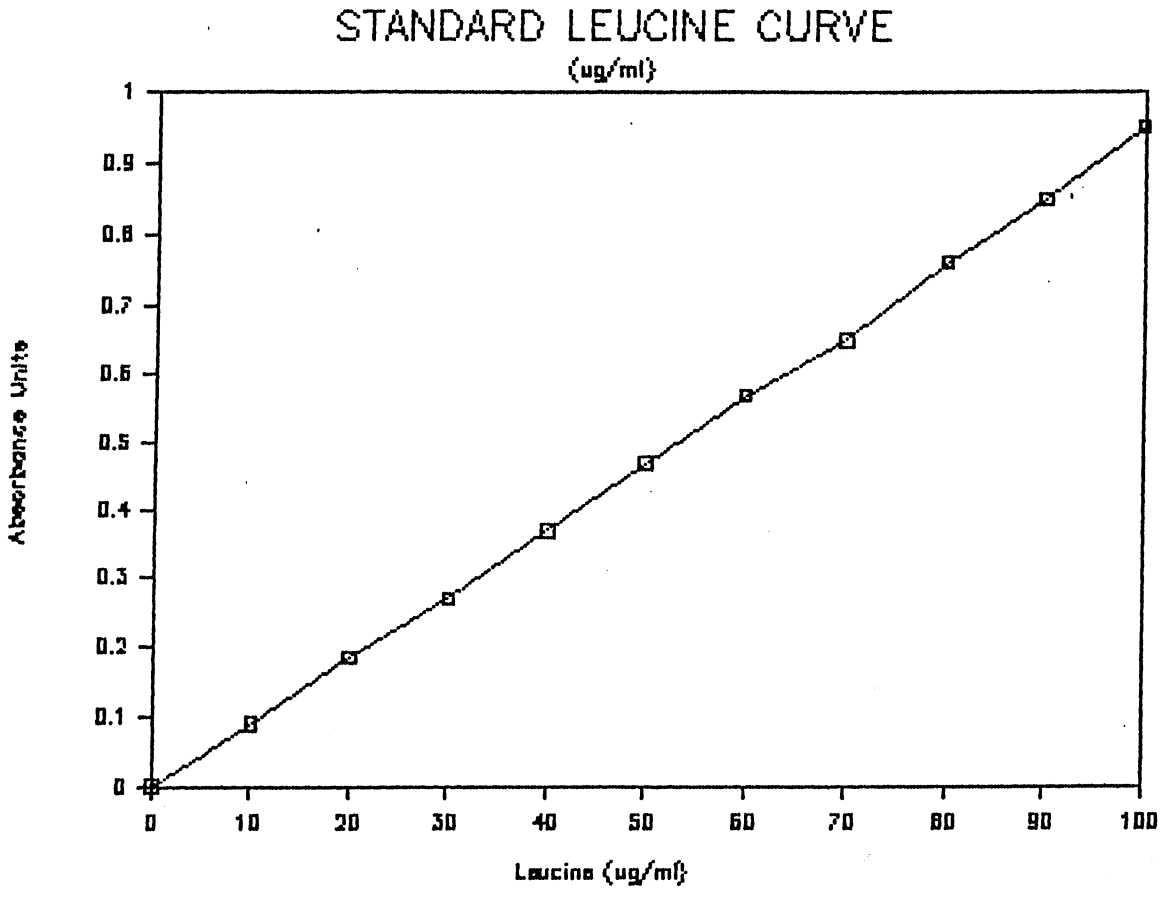


Figure C3. Standard calcium curve.

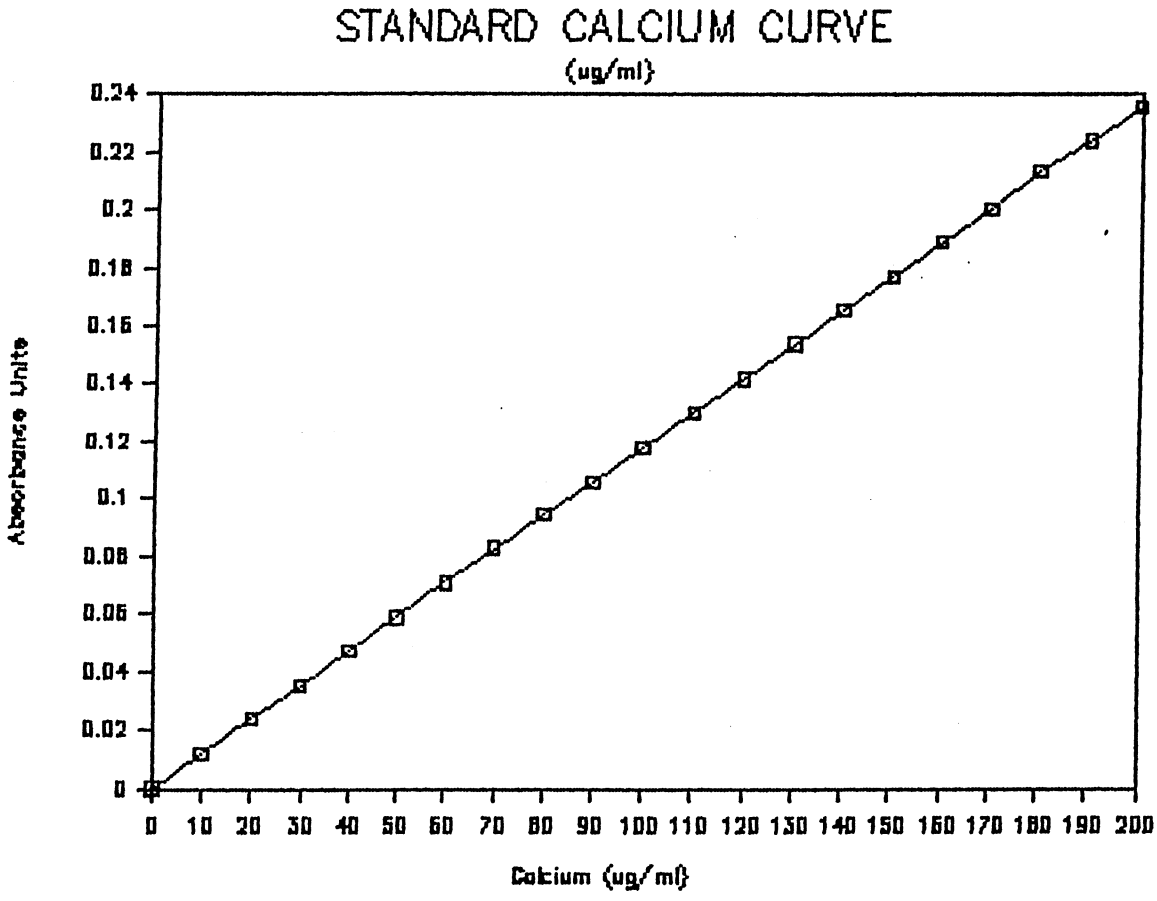
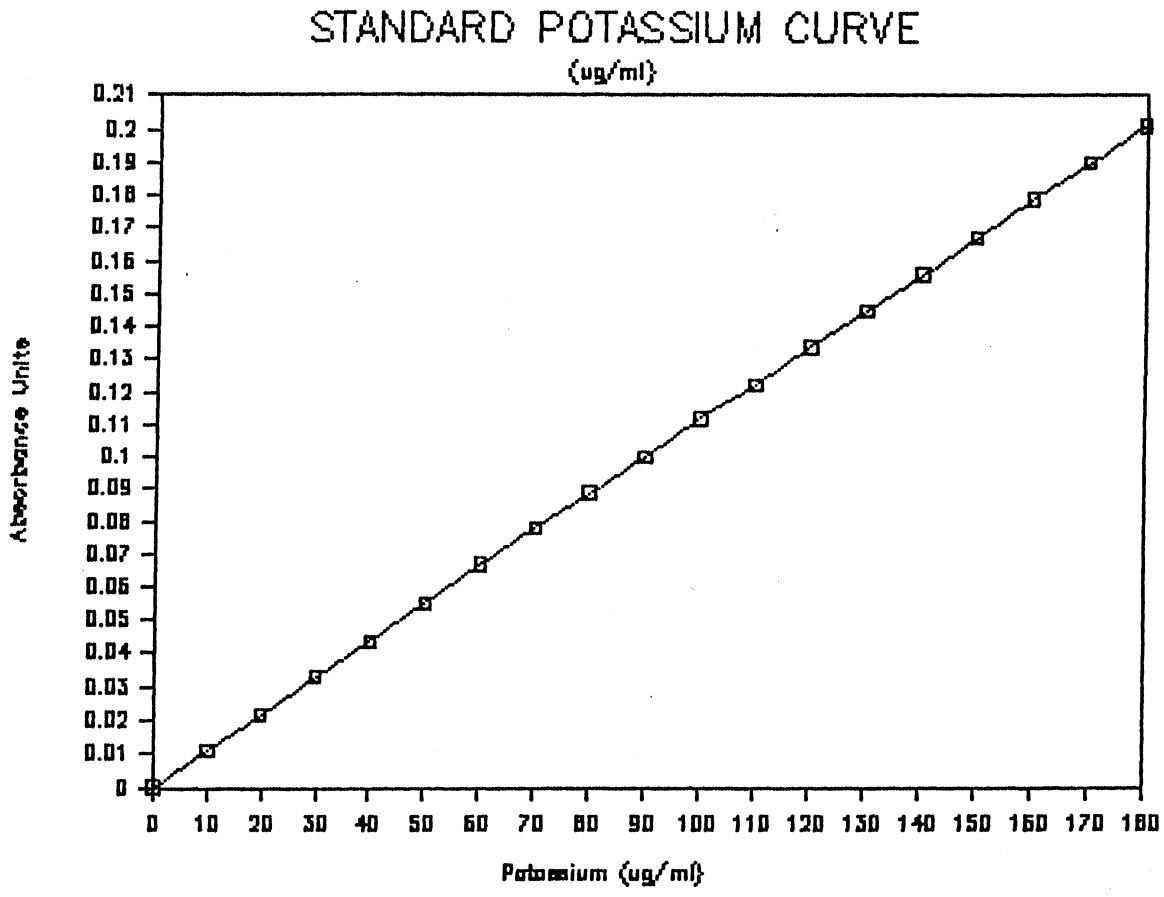


Figure C4. Standard potassium curve.



APPENDIX D

ANOVA TABLES

Table D1. Two-way analysis of variance for percent germination for cultivar by temperature.

Source	SS	df	MS	F	P	
Main Effects						
cult	9568.0555556	1	9568.0555556	287.04166667	.0000	***
temp	1811.1111111	2	905.55555556	27.166666667	.0000	***
Interaction						
cult x temp	144.44444444	2	72.222222222	2.1666666667	.1573	ns
Error	400	12	33.333333333			
Total	11923.611111	17				

Table D2. Two-way analysis of variance for days to 25% germination for cultivar by temperature.

Source	SS	df	MS	F	P	
Main Effects						
cult	30.680555556	1	30.680555556	32.485294118	.0001	***
temp	158.86111111	2	79.430555556	84.102941176	.0000	***
Interaction						
cult x temp	11.861111111	2	5.9305555556	6.2794117647	.0136	*
Error	11.333333333	12	0.9444444444			
Total	212.73611111	17				

Table D3. Two-way analysis of variance for percent ion leakage for cultivar by temperature.

Source	SS	df	MS	F	P	
Main Effects						
Cult	809.60266667	1	809.60266667	90.787887346	.0000	***
Temp	47.182333333	2	23.591166667	2.6454855819	.0802	ns
Interaction						
Cult x Temp	5.4863333333	2	2.7431666667	0.3076154718	.7365	ns
Error	481.546	54	8.9175185185			
Total	1343.8173333	59				

Table D4. Two-way analysis of variance for potassium in seed leachate for cultivar by temperature.

Source	SS	df	MS	F	P	
Main Effects						
Cult	0.017298	1	0.017298	323.3271028	.0000	***
Temp	0.0038041111	2	0.0019020556	35.552440291	.0000	***
Interaction						
Cult x Temp	2.47E-04	2	1.235E-04	2.308411215	.1418	ns
Error	6.42E-04	12	5.35E-05			
Total	0.0219911111	17				

Table D5. Two-way analysis of variance for electrical conductivity of seed leachate associated with analysis of potassium for cultivar by temperature.

Source	SS	df	MS	F	P	
Main Effects						
Cult	82.4328	1	82.4328	435.66574667	.0000	***
Temp	3.8453777778	2	1.9226888889	10.161606671	.0026	**
Interaction						
Cult x Temp	1.2724	2	0.6362	3.3623818192	.0693	ns
Error	2.2705333333	12	0.1892111111			
Total	89.821111111	17				

Table D6. Two-way analysis of variance for sugar in seed leachate for cultivar by temperature.

Source	SS	df	MS	F	P	
Main Effects						
Cult	20.358375	1	20.358375	31.023817755	.0000	***
Temp	1.009	2	0.5045	0.7687998702	.4686	ns
Interaction						
Cult x Temp	0.244	2	0.122	0.1859139428	.8309	ns
Error	35.43575	54	0.6562175926			
Total	57.047125	59				

Table D7. Two-way analysis of variance for electrical conductivity of seed leachate associated with analysis of sugars for cultivar by temperature.

Source	SS	df	MS	F	P	
Main Effects						
Cult	2760.8166667	1	2760.8166667	69.032529319	.0000	***
Temp	577.19433333	2	288.59716667	7.2161953417	.0017	**
Interaction						
Cult x Temp	60.876333333	2	30.438166667	0.7610877094	.4721	ns
Error	2159.621	54	39.992981481			
Total	5558.5083333	59				

Table D8. Two-way analysis of variance for amino acids in seed leachate for cultivar by temperature.

Source	SS	df	MS	F	P	
Main Effects						
Cult	79.534106667	1	79.534106667	215.5735552	.0000	***
Temp	0.5423233333	2	0.2711616667	0.7349712842	.4843	ns
Interaction						
Cult x Temp	0.2093433333	2	0.1046716667	0.2837077608	.7541	ns
Error	19.92286	54	0.3689418519			
Total	100.20863333	59				

Table D9. Two-way analysis of variance for electrical conductivity of seed leachate associated with analysis of amino acids for cultivar by temperature.

Source	SS	df	MS	F	P	
Main Effects						
Cult	66367.004167	1	66367.004167	189.19110865	.0000	***
Temp	4217.052	2	2108.526	6.0107334447	.0044	**
Interaction						
Cult x Temp	428.56533333	2	214.28266667	0.6108513678	.5466	ns
Error	18942.847	54	350.79346296			
Total	89955.4685	59				

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